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DYNAMIC SCREENING OF AGRICULTURAL CONTAMINANTS IN FRESHWATER ECOSYSTEMS AS PART OF AMPHIBIAN BIODIVERSITY CONSERVATION

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Introduction

In recent years the presence of agricultural contaminants in the aquatic environment has gained more interest. Research has shown negative effects on the fitness of aquatic amphibians and zooplankton as part of the aquatic food web. Furthermore, because most amphibians reside in aquatic habitats during breeding season and because they have a highly permeable skin, these species tend to be more exposed to environmental toxins than other aquatic and terrestrial vertebrates. Additionally, increasing anthropogenic pressure and agricultural pollution is affecting disease dynamics of chytridiomycosis in amphibians. The latter causing major population declines and even global extinction of species. In this study, a variety of a multi-residue analytical methods was applied for the simultaneous detection of pesticides (n=93), antimicrobial drug residues (n=46), mycotoxins (n=21), coccidiostats (n=12), heavy metals (n=8) and anthelmintics (n=3) in pond water resulting from 26 amphibian breeding ponds selected across Flanders, Belgium. Ponds were sampled monthly over a period of 4 months from March until June.

Objective

The overall objective of this study was to assess the level of contamination in amphibian breeding ponds and the general evolution over time.

Table 1: Overview of the 93 pesticides included in the screening method.
DDD* = dichlorodiphenyldichloroethane DDT* = dichlorodiphenyltrichloroethane DDE* = dichlorodiphenyldichloroethylene.

acephate	chlorpyrifos	ethoprophos	lindaan	pendimethanil	tebufenozide
acetamiprid	chlorthalonil	fenamiphos	linuron	pirimicarb	tebuthiuron
alachlor	cyflufenamid	fenbuconazole	malathion	prochloraz	temephos
aldrin	cymoxanil	fenitrothion	metalaxyl	profenofos	terbuthylazine
amethryn	cypermethrin	fenoxycarb	methiocarb	propanil	thiabendazole
azoxystrobine	difenconazole	fenpropimorf	methomyl	propiconazole	thiacloprid
bentazon	DDD*	fludioxonil	methoxychlor	propoxur	thiametoxam
bifenthrin	DDE*	heptachlorobenzeen	methsulfuron methyl	prosulfocarb	thifensulfuron
bitertanol	diazianon	hexachlorobenzeen	metribuzin	pyraclostrobin	thiodicarb
boscalid	dieldrin	hexaconazole	monocrotophos	pyrazosulfuron ethyl	thiofanate-methyl
butachlor	dimethoate	hexythiazox	nicosulfuron	pyrimethanil	triademinol
cadusafos	dimethomorph	imazalil	o,p'-DDT*	spirodiclofen	triazophos
captan	diuron	imidacloprid	oxamyl	spiroxamine	trifloxystrobine
carbaryl	endosulfan	iprodione	p,p'-DDT*	spinosad A	
carbendazim	endrin	kresoxim methyl	parathion	spinosad D	
carbofuran	epoxiconazole	lambda-cyhalothrin	penconazole	tebuconazole	

Table 2: Overview of the 46 antimicrobial drug residues included in the screening method. *diketopiperazine = amoxicillin-diketopiperazine-2',5'-dione

4-epichlortetracycline	florphenicol	sarafloxacin
4-epioxytetracycline	flumequine	sulfachlorpyridazine
4-epitetracycline	furaltadon	sulfadiazine
cefapirin	furazolidon	sulfadimethoxine
ceftiofur	lincomycin	sulfadoxine
cefquinome	marbofloxacin	sulfamethoxazole
chloramphenicol	nalidixic acid	sulfamerazine
chlortetracycline	nifursol	sulfamethazine
ciprofloxacin	nitrofurantoïn	sulfathiazole
cloxacillin	norfloxacin	tiamulin
danofloxacin	ofloxacin	tilmicosin
difloxacin	oxacillin	tetracycline
diketopiperazine*	oxolinic acid	trimethoprim
doxycycline	oxytetracycline	tylosin
enrofloxacin	penicillin G	
erythromycin A	penicillin V	

Table 3: Overview of the 21 mycotoxins included in the screening method.

15-acetyldeoxynivalenol	deoxynivalenol
3-acetyldexoxynivalenol	enniatin A
aflatoxin B1	enniatin A1
aflatoxin M1	enniatin B
alternariol methylether	enniatin B1
α-zearalanol	HT2-toxin
α-zearalenol	ochratoxin A
β-zearalanol	T2-toxin
β-zearalenol	zearalanone
beauvericin	zearalenone
deepoxy-deoxynivalenol	

Table 4: Overview of the 12 coccidiostats included in the screening method.

4,4'-dinitrocarbanilide	monensin
amprolium	narasin
diclazuril	robenidin
halofuginone	salinomycin
lasalocid	semduramicin
maduramicin	toltrazuril

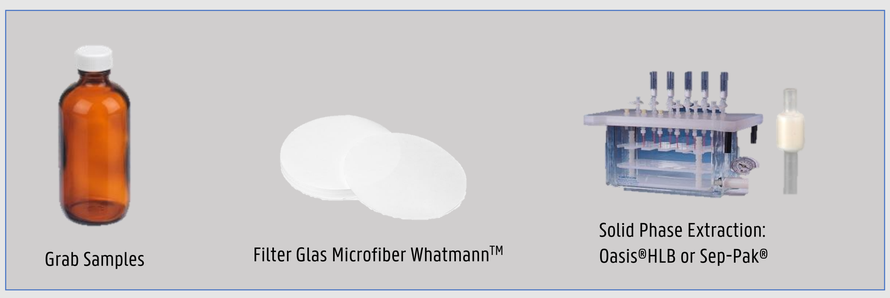
Table 5: Overview of the 8 heavy metals included in the screening method.

arsenic	lead
cadmium	mercury
chromium	nickel
copper	zinc

Table 6: Overview of the 3 anthelmintics included in the screening method.

flubendazole
ivermectin
levamisole

Methods



Results Field Sampling

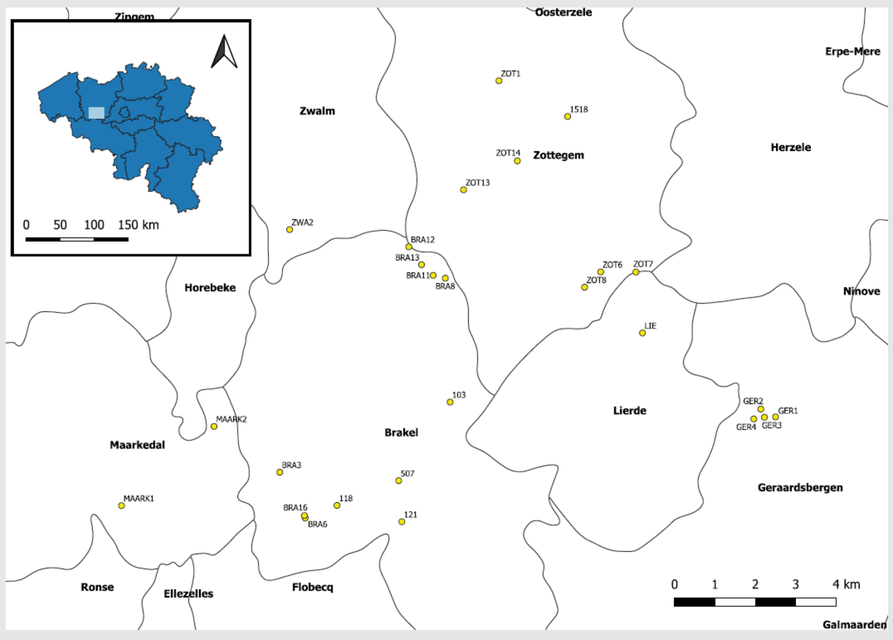


Fig. 1: Sampling locations selected across East Flanders, Belgium including ponds in Zottegem (n=7), Brakel (n=11), Lierde (n=1), Geraardsbergen (n=4) , Maarkedal (n=2) and Zwalm (n=1).

Table 7: Contaminants detected in pond water during the sampling campaign of March untill June 2019.

Class	Concentration range (ng mL^{-1})	Most abundant compound
Heavy metals	0.206-333	zinc
Antimicrobial drug residues	0.003-0.422	4-epioxytetracycline
Pesticides	0.002-38.7	terbuthylazine
Mycotoxins	0.001-0.007	enniatin B
Coccidiostats	0.005-0.029	amprolium
Anthelmintics	0.003-5.700	levamisole

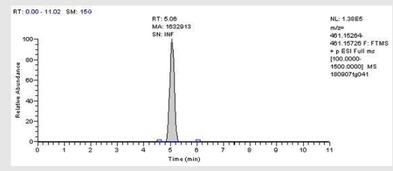


Fig. 3: 4-epioxytetracycline detection in pond BRA12 (Brakel, Belgium).

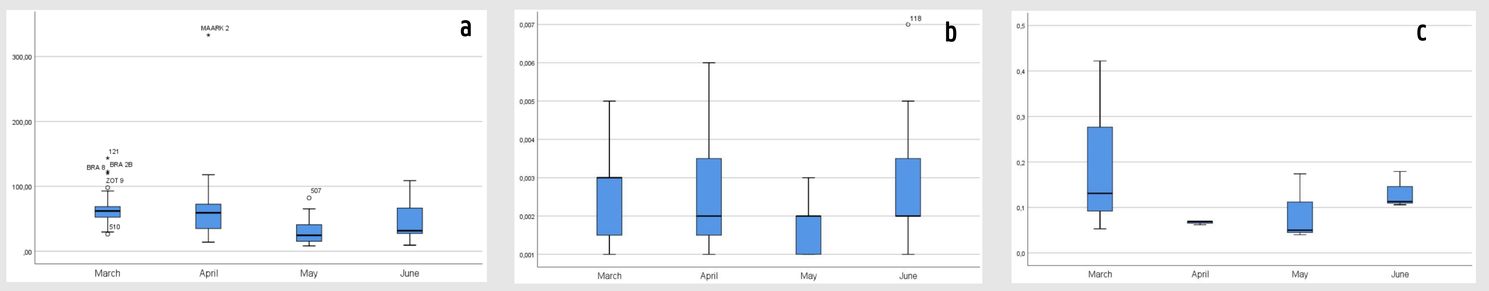


Fig. 2: Box-plots of the concentration range (ng mL^{-1}) of zinc (a), enniatin B (b) and 4-epixoxytetracycline (c) during the months of March, April, May and June. Within the box plot chart the crosspieces of each box plot represent (from top to the bottom) maximum, upper-quartile, median (black bar), lower-quartile and minimum values.

General conclusion

A variety of multi-residue analytical methods was applied for the simultaneous detection of pesticides (n=93), antimicrobial drug residues (n=46), mycotoxins (n=21), coccidiostats (n=12) heavy metals (n=8) and anthelmintics (n=3) in pond water resulting from 26 amphibian breeding ponds selected across Flanders, Belgium. Most abundant compounds for each group of contaminants were zinc (C_{max} = 333 ng mL^{-1}), terbuthylazine (C_{max} = 39 ng mL^{-1}), levamisole (C_{max} = 5.7 ng mL^{-1}), 4-epioxytetracycline (C_{max} = 0.4 ng mL^{-1}), amprolium (C_{max} = 0.03 ng mL^{-1}) and enniatin B (C_{max}= 0.01 ng mL^{-1}). Overall, the concentrations of enniatin B and 4-epioxytetracycline were relatively stable during the sampling period. Zinc concentrations were significantly lower in May in comparison with March, April and June: 30 versus 62, 66 and 45 ng mL^{-1}, respectively (with a p-value of < 0.05). These findings could be related to a reduced application of pig manure, associated with higher zinc concentrations, on the surrounding agricultural fields in combination with higher precipitation in May.

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